Genetic diversity of two tropical tree species of the Dipterocarpaceae following logging and restoration in Borneo: high genetic diversity in plots with high species diversity

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Abstract: Background: The impact of logging and restoration on species diversity has been well studied in tropical forests. However, little is known about their effects on genetic diversity within species. Aims: We assess the degree of genetic diversity among dipterocarp seedlings used for enrichment planting of selectively logged forests in Sabah, Malaysia, and compare it with diversity in naturally regenerating seedlings. Methods: We sampled young leaf tissues from seedlings of Shorea leprosula and Parashorea malaanonan for DNA genotyping, using microsatellite markers. Results: The levels of genetic diversity (expected heterozygosity and rarefied allelic richness) of naturally regenerating seedlings were statistically indistinguishable among unlogged, once logged and repeatedly logged forest areas. Enrichment-planted seedlings of P. malaanonan exhibited similar levels of genetic diversity to naturally regenerating seedlings whereas those of S. leprosula had significantly lower genetic diversity than natural seedlings. Interestingly, reduction of genetic variation was consistently observed in single-species plots relative to mixed-species plots among enrichment-planted seedlings. Conclusions: There was no reduction of genetic variation in naturally regenerating dipterocarp seedlings in areas of selective logging. However, genetic variation of enrichment-planted seedlings was lower in single-species plots relative to mixed-species plots. This suggests that enrichment-planting strategies should adopt diverse mixtures that should promote levels of both species richness and genetic diversity within species.

DOI: https://doi.org/10.1080/17550874.2016.1270363

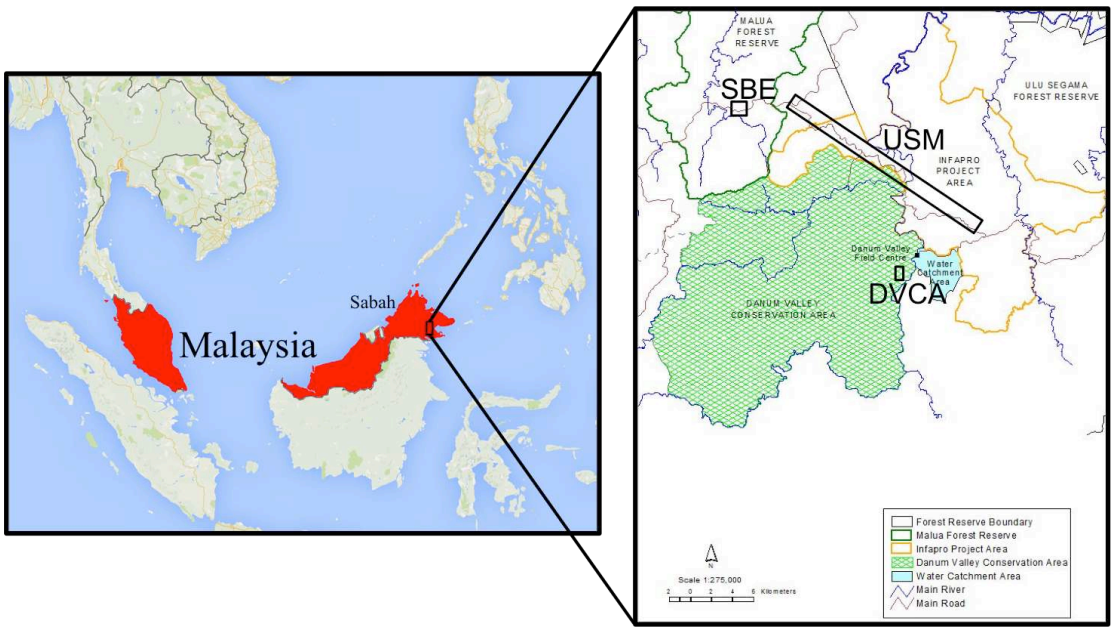

Figure S1. Map of Malaysia (red) and the three study sites (marked in black). The Sabah Biodiversity Experiment (SBE), provided a less intensively logged forest and sampling of enrichment-planted seedlings. The Ulu Segama Malua Forest Reserve (USM) provided an intensively logged forest, and the Danum Valley Conservation Area (DVCA) provided an unlogged forest (sources from Yayasan Sabah Group).

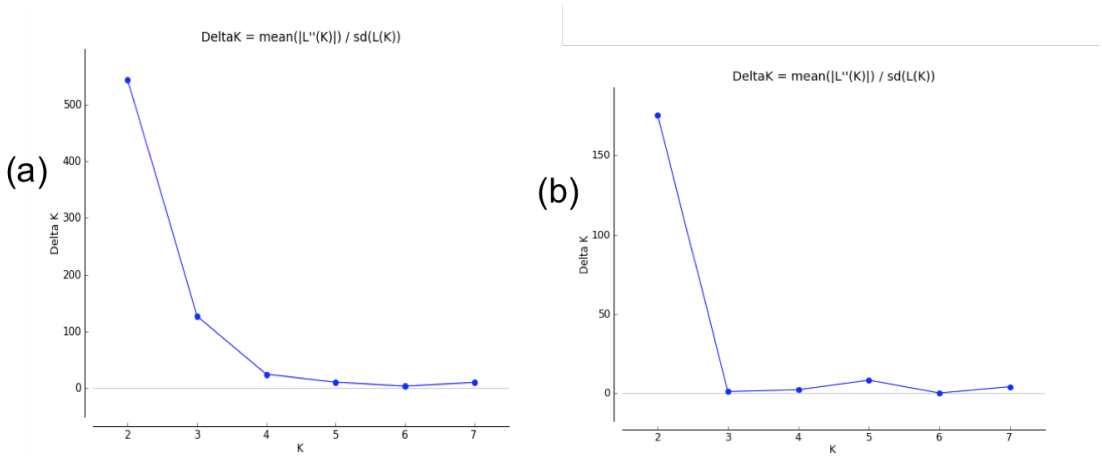

Figure S2. *ΔK* of (a) *Shorea leprosula* (with 14 microsatellite loci and six pre-assigned locations) and (b) *Parashorea malaanonan* (with eight microsatellite loci and six pre-assigned locations) at three sites under different management or restoration strategies in Sabah, Malaysia.

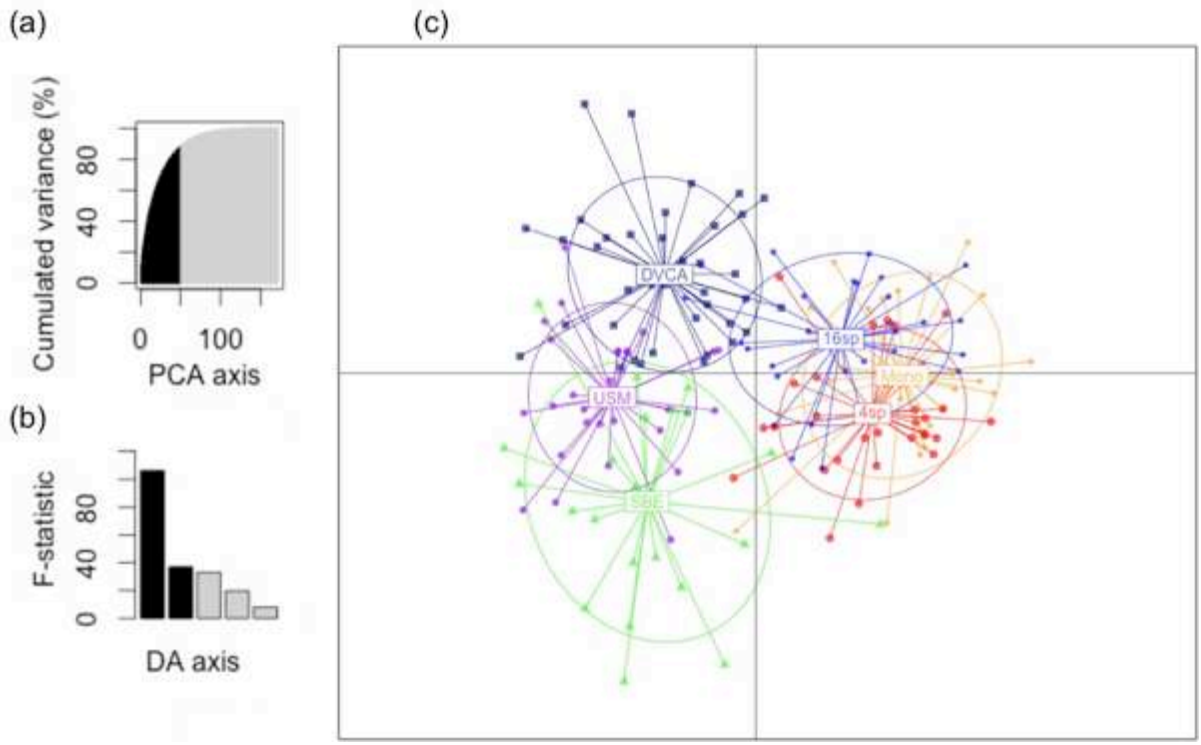

Figure S3. DAPC for *Shorea leprosula* at three sites under different management or restoration strategies in Sabah, Malaysia. (a) Variance explained by PCA in DAPC: >80% of the total microsatellite genetic variability was explained by retaining the first 50 PCA components (in black). (b) Bar plot of eigenvalues for the discriminant analysis (first two axes in black) with *F*-statistic values. (c) Scatter plot of *S. leprosula* on the first two axes of DAPC. The scatter plot supported the $K = 2$ hypothesis, where one cluster comprised natural seedlings, while another cluster was formed by closely related planted seedlings. However, a small overlapping of DVAC with 16-species plots was observed, unexpectedly.

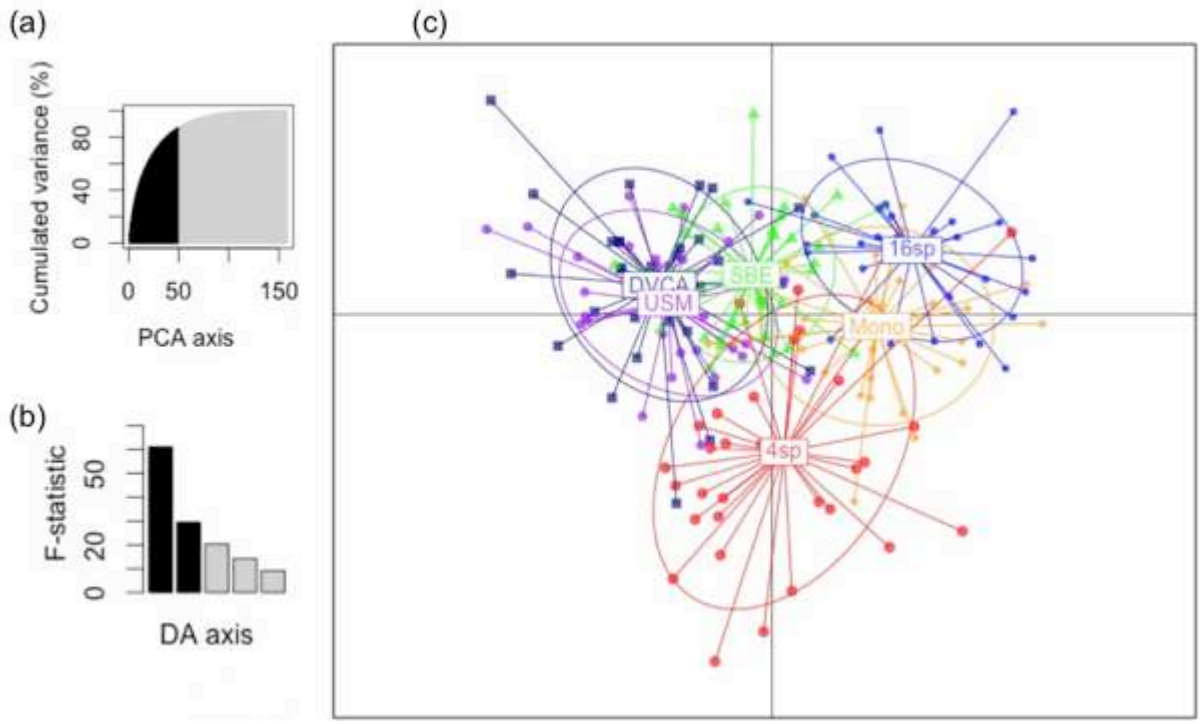

Figure S4. DAPC for *Parashorea malaanonan* at three sites under different management or restoration strategies in Sabah, Malaysia. (a) Variance explained by PCA in DAPC: >80% of the total microsatellite genetic variability was explained by retaining the first 50 PCA components (in black). (b) Bar plot of eigenvalues for the discriminant analysis (first two axes in black) with *F*-statistic values. (c) Scatter plot of *P. malaanonan* on the first two axes of DAPC. The scatter plot does not explicitly display a differentiation between natural and planted seedlings.

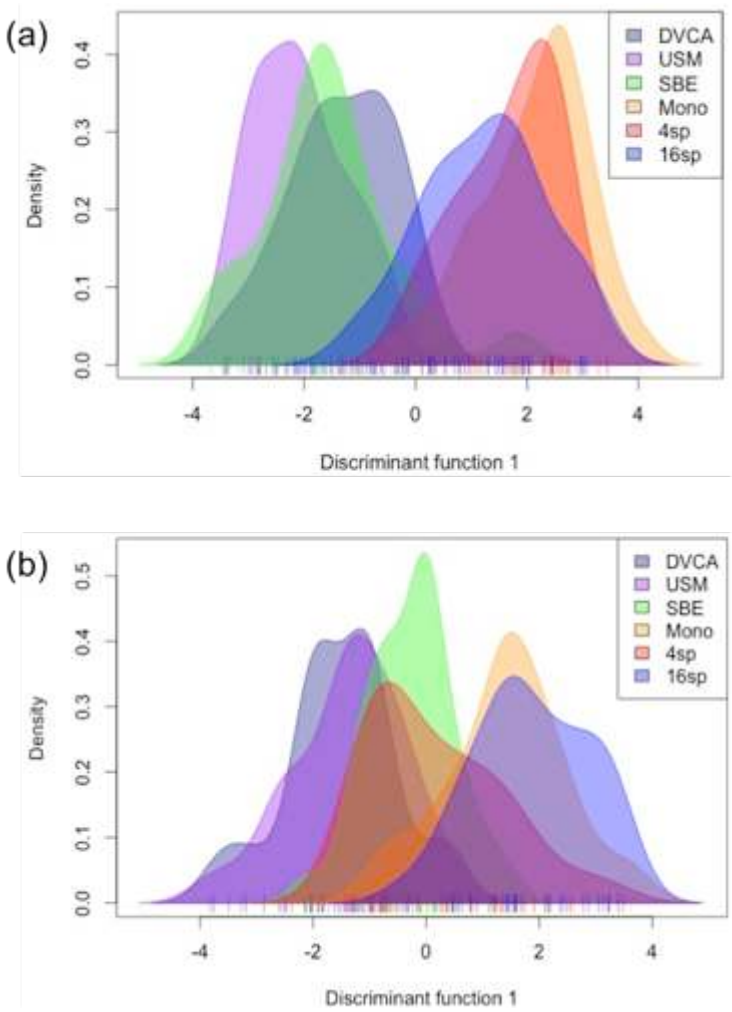

Figure S5. Density plot generated from a single discriminant function of (a) *Shorea leprosula* and (b) *Parashorea malaanonan* from the three sites under different management or restoration strategies in Sabah, Malaysia. Distinctive genetic clusters are observed in (a), inferring $K = 2$ among all the 183 seedlings of *S. leprosula.* In contrast, an ambiguous relationship was observed between groups of *P. malaanonan* seedlings in (b).

Table S1. Set of 14 nuclear microsatellite loci used in the genotyping of *Shorea leprosula* seedlings (Lee et al. 2004) at three sites under different management or restoration strategies in Sabah, Malaysia.

Locus	**Forward (5′→3′)**	**Reverse (5′→3′)**	**Repeat**	**Accession number**
Sle074a	ATCACCAAGTACCTATCATCA	GCAATGGCACACAGTCTATC	$(CT)_{11}$	AJ616872
Sle079	GTTGTCTGTTCTTACCAGGAAG	GCATAAGTATCGTCGCCA	$(CT)_{11}$	AJ616873
Sle105	CTGTGTCAAAATCAGTTAGGACTTACGAG	GAGTCGATTGCTTGTCTTCACCC	$(GA)_{12}$	AJ616874
Sle111a	GGAAACTACTGGAGCAGAGAC	GGTGGGTTATGGAGAATGAG	$(GA)_{14}$	AJ616875
Sle118	AAAGCGTACAAATTCATCA	CTATTGGTTGGGTCAGAAGG	$(GA)_{16}$	AJ616876
Sle267	CTTAATTGTGATGCCTGTTG	TCTTGTATTTATGCTTCTCC	$(GA)_{17}$	AJ616878
Sle271a	CAGGAACCGACTATCTGCC	TCATTCAAATCAAACTAATTTC	$(GA)_{7}$	AJ616879
Sle280	GCAACTAAAATGGACCAGA	GAGTAAGGTGGCAGATATAGAG	$(CT)_{7}$	AJ616880
Sle294	AACTAAATGTAAAATCTTCC	CTTTTGAGATATAATGTTGA	$(GA)_{12}$	AJ616883
Sle303a	TCCTTACATGGACTGAGATTCACC	GTTTCAATTATGAGGGGAACTGATTTAC	$(GA)_{12}$	AJ616884
Sle384	CCAAGACAACTCAATCCTCA	AGATGAAGGTGTTGCTGTG	$(CT)_{13}$	AJ616885
Sle475	AGCGAAACCCTTGTGGAGA	GAGACTACGGTGGCGACGA	$(GA)_{10}$	AJ616888
Sle562	TGATTTGGGTGGTTGTAG	TATTACATTTTTCAAGTCAAGTC	$(GT)_{8}$	AJ616889
Sle605	GTGCATTTATTGCCTGAGTAAGGTGG	CAACTAAAATGGACCAGACCGGATG	$(GA)_{13}$	AJ616891

Table S2. Set of eight nuclear microsatellite loci used in the genotyping of *Parashorea malaanonan* seedlings (Lee et al. 2004; Ujino et al. 1998) at three sites under different management or restoration strategies in Sabah, Malaysia.

Locus	**Forward (5′→3′v)**	**Reverse (5′→3′)**	**Repeat**
Shc01	GCTATTGGCAAGGATGTTCA	CTTATGAGATCAATTTGACAG	$(CT)_8(CA)_{10}CT(CA)_4CTCA$
Shc04	ATGAGTAACAAGTGATGAG	TATTGACGTGGAATCTG	$(CT)_{16}$
Shc07	ATGTCCATGTTTGAGTG	CATGGACATAAGTGGAG	$(CT)_8CA(CT)_5CACCC(CTCA)_3CT(CA)_{10}$
Shc11	ATCTGTTCTTCTACAAGCC	TTAGAACTTGAGTCAGATAC	$(CT)_4TT(CT)_5$
Sle074a	ATCACCAAGTACCTATCATCA	GCAATGGCACACAGTCTATC	$(CT)_{11}$
Sle105	CTGTGTCAAAATCAGTTAGGACTTACGAG	GAGTCGATTGCTTGTCTTCACCC	$(GA)_{12}$
Sle267	CTTAATTGTGATGCCTGTTG	TCTTGTATTTATGCTTCTCC	$(GA)_{17}$
Sle562	TGATTTGGGTGGTTGTAG	TATTACATTTTTCAAGTCAAGTC	$(GT)_8$

Table S3. Pairwise F_{ST} genetic distance two tree species in the Dipterocarpaceae family at three sites at three sites, Danum Valley Conservation Area (DVCA), Ulu Segama Malua Forest Reserve (USM) or the Sabah Biodiversity Experiment (SBE) under different levels of species richness of the enrichment plantings (i.e. Mono: one species, 4sp: four species or 16sp: 16 species) in Sabah, Malaysia.

	Shorea leprosula					*Parashorea malaanonan*				
	DVCA	**USM**	**SBE**	**Mono**	**4sp**	**DVCA**	**USM**	**SBE**	**Mono**	**4sp**
DVCA										
USM	0.0058					0.0054				
SBE	0.0087	0.0039				0.0033	0.0105			
Mono	0.0492	0.0544	0.0469			0.0220	0.0263	0.0086		
4sp	0.0633	0.0643	0.0515	0.0075		0.0106	0.0138	0.0095	0.0161	
16sp	0.0227	0.0378	0.0298	0.0527	0.0943	0.0229	0.0210	0.0149	0.0213	0.0170